

THE MINIMAL INFECTIOUS DOSE OF ADENOVIRUS TYPE 4; THE CASE FOR NATURAL TRANSMISSION BY VIRAL AEROSOL

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Acute respiratory disease of military recruits, designated ARD, was defined as a clinical entity before the discovery of its etiology, now known to be most commonly adenovirus type 4.¹ It is an acute febrile illness characterized by cough, hoarseness, sore throat, and constitutional symptoms, and occurs in epidemics usually in January, February, and March. ARD may account for more than 25 per cent of the annual occurrence of respiratory illness in recruits. Despite the apparent ease of contracting the disease, as demonstrated by the occurrence of epidemics, early experimental studies showed that nasal inoculation of volunteers with several serotypes of adenovirus seldom resulted in illness, although asymptomatic nasopharyngeal and intestinal infections were easily produced.

In an effort to discover an experimental method for regular production of illness similar to the natural disease, studies were made in volunteers of small particle aerosol inoculation with adenovirus type 4. For comparison, similar studies were made by nasal inoculation. The aerosol used had a median diameter of 1.5 μ , a size which deposits primarily in the lower respiratory tract. However, a portion of these particles, because of their size, will not deposit but will be exhaled.

Nasal inoculation deposits virus direct on the membranes of the nasopharynx. It is possible, however, that some may become resuspended from mucus membranes by forceful inspiration and be carried down into the lung.

METHODS

The source and procedures for use of volunteers, preparation of inoculum, preparation and use of aerosol, virus isolation, virus identification

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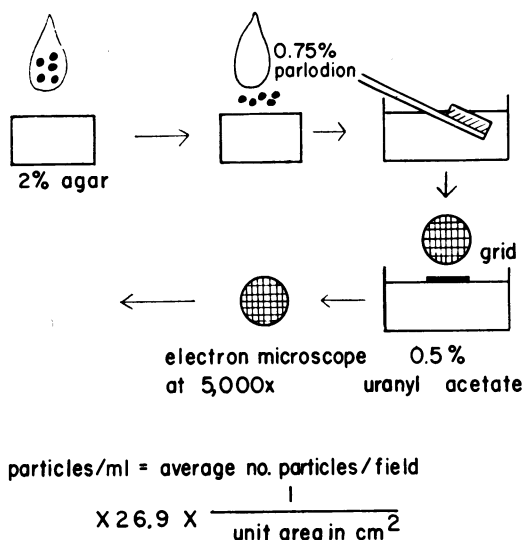


Fig. 1. Method of preparation of adenovirus type 4 inoculum for quantitative electron microscopic counting.

and serologic tests are described in a previous report.² Electron microscopic methods were modified from procedures described by Smith and Benyesh-Melnick³ and Smith and Melnick⁴ as follows: A drop of viral inoculum was placed on an agar block where it spread and dried in an area roughly equivalent to 1 cm² (Figure 1). The area was coated with a film of parlodion which, after drying, was floated onto 0.5% uranyl acetate. The parlodion film with attached virus was then placed on an electron microscope grid and examined at 5000 \times magnification. Five randomly selected fields were photographed, counted, and averaged for the total number of viral particulates per field. They were also characterized for the number of viruses (virions) in each particulate, and the frequency of empty particles, which would suggest nonviability. The concentration of viral particulates was calculated by the formula at the bottom of the figure.

In two series of five fields each, counted on material from the same vial of inoculum, the mean number of single virions per field was 65 (range 42–91, SD \pm 14). The smaller values for counts of aggregates of virus showed more variable results.

RESULTS

Human Infectious Dose. The results of a study of nasal inoculation of volunteers selected as free of serum antibody to adenovirus type 4 are

TABLE I
*Response of Volunteers Free of Antibody to Nasal Inoculation
with Adenovirus Type 4*

Adenovirus 4 Nasal Inoculation (TCID ₅₀)	Number of Volunteers	Number Infected	Number Ill
400	3	3	0
79	3	2	1
14	2	1	0
10	2	0	0
3	6	0	1

HID₅₀ = 35 TCID₅₀ (95% limits 8-157).

TABLE II
*Response of Volunteers Free of Antibody to Aerosol Inoculation
with Adenovirus Type 4*

Adenovirus 4 1.5 μ Aerosol Dose (TCID ₅₀)	Number of Volunteers	Number Infected	Number Ill
171	4	4	4
5-11	9	9	8
1-2	5	3	3
0.1	3	0	0

HID₅₀ = 0.5 TCID₅₀ (95% limits 0.2-1.4).

[Data on 9 subjects who received 5-11 TCID₅₀ are from reference 2.]

shown in Table I. Infection occurred in six of 16 volunteers, and the lowest dose causing infection was fourteen 50 per cent tissue culture infectious doses (TCID₅₀). The 50 per cent human infectious dose (HID₅₀) by nasal inoculation was 35 TCID₅₀. Illness occurred in two men but was associated with adenovirus infection only in the volunteer who was given 29 TCID₅₀ intranasally. The other illness was not etiologically identified. Thus, only one of six infected men developed illness with adenovirus.

In contrast, aerosol inoculation of 21 volunteers (Table II) in doses ranging from 0.1 to 171 TCID₅₀ caused infection in 16 and illness in 15. The HID₅₀ by the aerosol route was 0.5 TCID₅₀. The size of dose was not related to severity of illness. Once illness occurred, it was as likely to be severe with a low as with a high dose. Results of inoculation of a volunteer with 7 TCID₅₀ by small particle aerosol are shown in Figure 2. Illness began on the fifth day after inoculation although respiratory secretions and an anal swab yielded low titers of virus on the third day. Illness was characterized by fever up to 40°C, sore throat, cough, and

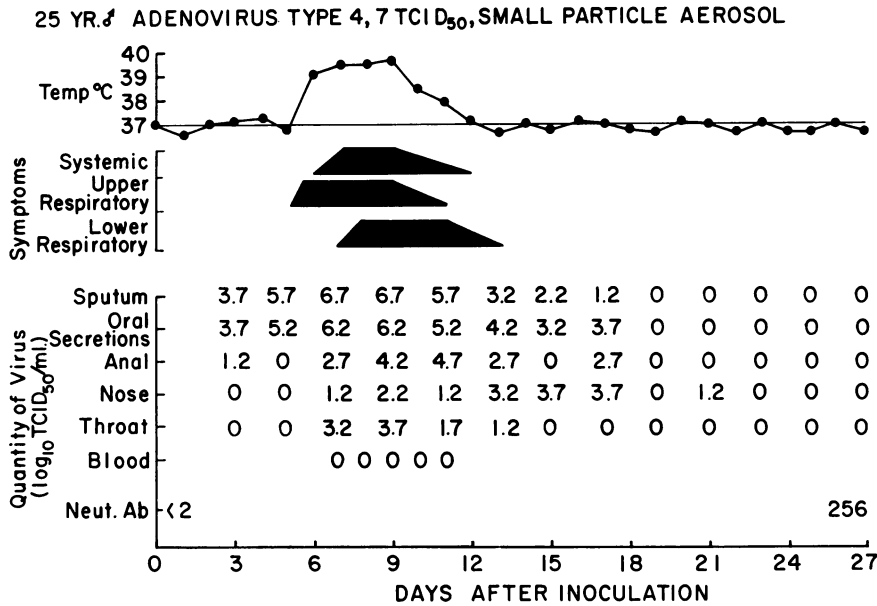


FIG. 2. Results of inoculation of a volunteer with 7 TCID₅₀ by small particle aerosol.

prostration, and lasted for a week. Virus titers in respiratory secretions reached 10^{6.7} per ml during the peak period of illness. Anal shedding persisted for three weeks after inoculation. Several blood cultures were negative. In another volunteer (not shown) who received 11 TCID₅₀ by small particle aerosol, mild left lower lobe pneumonia occurred, thus completing a demonstration of the syndromes of illness which characterize adenovirus disease in recruits.

Enumeration of Viral Particulates by Electron Microscopy. In view of the remarkable efficiency of low doses of small particle adenovirus aerosol in the production of acute respiratory disease, it became of interest to characterize the inoculum in terms of the numbers of viral particulates necessary to cause the disease. In the study, suspensions of virus whose human infectivity (HID₅₀) by aerosol and nasal inoculation was known in terms of tissue culture infectivity (TCID₅₀), were examined quantitatively by electron microscopy. Table III shows the results of this examination. Of greatest interest is the fact that an average of 6.6 viral particulates by aerosol was sufficient to infect 50 per cent of susceptible volunteers. When it is recalled that a portion of the dose will be exhaled, and that two thirds of the viral particulates are single virions, the infectious dose of adenovirus for man by small particle aerosol is exceedingly small. In contrast, about 462 particulates were required by

TABLE III
Distribution of Virions in Particulates of Adenovirus Type 4 Inoculum

Indicated Number of Virions	Percentage of Particulates in Inoculum	Number of Particulates with Indicated Number of Virions in 1 HID ₅₀	
		Nasal (35 TCID ₅₀)	Aerosol (0.5 TCID ₅₀)
1 virion	67%	310	4.4
2 virions	11	51	0.7
3 “	8	37	0.5
4 “	4	18	0.3
5 “	3	14	0.2
6+ “	7	32	0.5
Total	100%	462	6.6

1 ml of virus suspension contained 1.1×10^8 TCID₅₀ or 1.5×10^9 viral particulates.

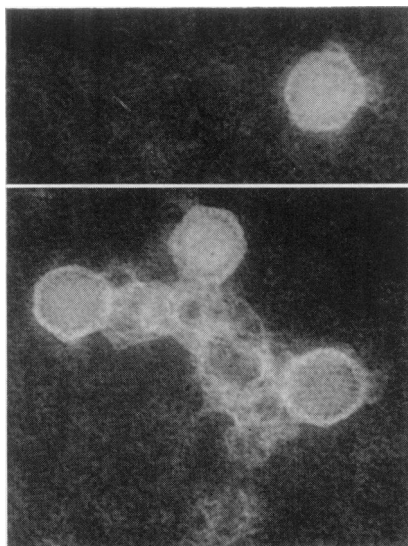


FIG. 3. Adenovirus type 4 inoculum, 40,000 \times . Single virion and a particulate with 3 virions.

the nasal route to produce infection and infrequent illness. Empty virions constituted less than 3 per cent of the total count and are not included in this table.

A photograph made at 40,000 \times of a portion of one of the fields on which counts were made is shown in Figure 3. One particle is a single intact icosahedron of adenovirus; the other is a cluster of 3 or 4 virions attached to some detritus.

DISCUSSION AND SUMMARY

These studies have revealed that a few particles of adenovirus type 4 may cause human illness when administered as a small particle aerosol. This indicates an extreme susceptibility of cells in the lower respiratory tract to infection by this agent, and the acute systemic illness may possibly indicate systemic spread of the infection. It is reasonable to suspect that some of the soluble subunits of the protein coat of the virus (which consist of hexons, pentons, and fibers) that are produced in great excess in tissue culture are also produced in the lung and act as noninfectious irritants to cause systemic reaction, as well as to stimulate protective antibody. We have on a number of occasions attempted to isolate adenovirus from the blood of volunteers but have so far been unsuccessful, although viremia has been described for naturally occurring cases.⁵

In contrast to the exquisitely susceptible cells of the lower respiratory tract, an approximately 70-fold greater dose was required to cause infection in the nasopharynx, and illness occurred infrequently. The intestinal infection as measured by rectal shedding probably is produced by swallowed virus, and also remains localized. This finding is the basis for the use of enteric, coated capsules of live virus for vaccination.⁶

In view of the apparent requirement that inoculation of the *lower* respiratory tract is necessary for development of most acute illness (ARD) in volunteers, we propose this mechanism for the natural occurrence of ARD in recruits. For this to occur, infected persons would have to produce by coughs and sneezes and other exhalations sufficient amount of infectious small particle aerosols to produce infection. Studies in this laboratory have shown that coughs and sneezes do produce such particles, and we have recovered an enterovirus from coughs and from the air of rooms occupied by volunteers infected with this agent.⁷ Artenstein and Miller have isolated adenovirus from the air of a military barracks occupied by recruits with acute respiratory disease.⁸

If further studies confirm that small particle aerosol plays a major role in the natural transmission of ARD, as suggested by the present studies in volunteers, then purification of air in barracks rooms and other places where recruits are in close contact, should diminish the spread of these infections.

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